\*This protocol was modified from QIAamp Fast DNA Stool Mini Handbook and kindly provided by Dr. Yen-Hsuan Ni (倪衍玄)

1. **檢體以冷凍運送至實驗室**
2. **檢體分裝至2ml離心管，並放置於-80℃保存。**

**Protocol: Isolation of DNA from Stool for Pathogen Detection**

Lysis conditions in this protocol are optimized to increase the ratio of nonhuman DNA to human DNA. Human DNA is not excluded by this protocol.

**Important points before starting**

* All centrifugation steps should be carried out at room temperature (15–25°C) at 20,000 x g (approximately 14,000 rpm). Increase the centrifugation time proportionately if your centrifuge cannot provide 20,000 x g (e.g., instead of centrifuging for 5 min at 20,000 x g, centrifuge for 10 min at 10,000 x g).

**Things to do before starting**

◎ Prepare block heaters or water baths at 95°C and 70°C for use in steps 3 and 8

◎ Redissolve any precipitates in Buffer AL and InhibitEX Buffer by incubating at 37–70°C

◎ Add ethanol to Buffer AW1 and Buffer AW2 concentrates

◎ Mix all buffers before use

**Procedure**

**1. Weigh 180–220 mg stool in a 2 ml microcentrifuge tube and place tube on ice.**

If the sample is liquid, pipet 200 μl into the microcentrifuge tube. Cut the end of the pipet tip to make pipetting easier. If the sample is frozen, use a scalpel or spatula to scrape bits of stool into a 2 ml microcentrifuge tube on ice.

Note: When using frozen stool samples, take care that the samples do not thaw until InhibitEX Buffer is added in step 2 to lyse the sample; otherwise the DNA in the sample may degrade. After addition of InhibitEX Buffer, all following steps can be performed at room temperature (15–25°C).

**2. Add 1 ml InhibitEX Buffer to each stool sample.**

Vortex continuously for 1 min or until the stool sample is thoroughly homogenized.

Note: It is important to vortex the samples thoroughly. This helps ensure maximum DNA concentration in the final eluate.

**3. Heat the suspension for 5 min at 95°C. Vortex for 15 s.**

This heating step helps to lyse bacteria and other parasites. The lysis temperature can be increased to 95°C for cells that are difficult to lyse (such as Gram-positive bacteria).

**4. Centrifuge sample at full speed for 1 min to pellet stool particles.**

IMPORTANT: Do not transfer any solid material. If particles are still visible in the supernatant, centrifuge the sample again.

**5. Pipet 30 µl proteinase K into a new 1.5 ml microcentrifuge tube (not provided). 6. Pipet 400 µl supernatant from step 4 into the 1.5 ml microcentrifuge tube containing proteinase K.**

**7. Add 400 µl Buffer AL and vortex for 15 s.**

Note: Do not add proteinase K directly to Buffer AL. It is essential that the sample and Buffer AL are thoroughly mixed to form a homogeneous solution.

**8. Incubate at 70°C for 10 min.**

Centrifuge briefly to remove drops from the inside of the tube lid (optional).

**9. Add 400 µl of ethanol (96–100%) to the lysate, and mix by vortexing.**

Centrifuge briefly to remove drops from the inside of the tube lid (optional).

**10. Carefully apply 600 µl lysate from step 9 to the QIAamp spin column**. **Twice**

Close the cap and centrifuge at full speed for 1 min. Place the QIAamp spin column in a new 2 ml collection tube, and discard the tube containing the filtrate. Close each spin column in order to avoid aerosol formation during centrifugation. If the lysate has not completely passed through the column after centrifugation, centrifuge again until the QIAamp spin column is empty.

**11. Carefully open the QIAamp spin column and add 500 µl Buffer AW1.**

Centrifuge at full speed for 1 min. Place the QIAamp spin column in a new 2 ml collection tube, and discard the collection tube containing the filtrate.

**12. Carefully open the QIAamp spin column and add 500 µl Buffer AW2.**

Centrifuge at full speed for 3 min. Discard the collection tube containing the filtrate. Note: Residual Buffer AW2 in the eluate may cause problems in downstream applications. Some centrifuge rotors may vibrate upon deceleration, causing the flow-through containing Buffer AW2 to come in contact with the QIAamp spin column. Removing the QIAamp spin column and collection tube from the rotor may also cause flow-through to come into contact with the QIAamp spin column.

**13. Place the QIAamp spin column in a new 2 ml collection tube (not provided) and discard the old collection tube with the filtrate. Centrifuge at full speed for 3 min.**

This step helps to eliminate the chance of possible Buffer AW2 carryover.

**14. Transfer the QIAamp spin column into a new, labeled 1.5 ml microcentrifuge tube (not provided) and pipet 50 µl ddH2O directly onto the QIAamp membrane. Incubate for 1 min at room temperature, then centrifuge at full speed for 1 min to elute DNA**.

For long-term storage, we recommend keeping the eluate at –20°C.